Efficacy assessment of ultraviolet germicidal irradiation (UVGI) devices for inactivating airborne *Mycobacterium tuberculosis*

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ABSTRACT

Introduction: Airborne transmission of *Mycobacterium tuberculosis* (TB) and other infectious agents within indoor environments has been a recognised hazard for decades. The increasing incidence of airborne diseases and drug resistance has renewed interest in ultraviolet germicidal irradiation (UVGI) to reduce transmission. The aim of this study was to determine the efficacy of UVGI devices, available in South Africa, for inactivating airborne TB bacteria.

Methodology: Thirteen UVGI devices from major South African suppliers were challenged with *M. tuberculosis* H37Ra bacilli (~1 x 10^6 vegetative cells/ml) when OFF and when ON, for one hour. Air samples (n = 130) were collected using PTFE filters. Sample extracts were analysed using quantitative real time polymerase chain reaction (qPCR), targeting the 16 subunit ribosomal ribonucleic acid (16S rRNA) gene. The DNA extraction efficiency was also determined, using the Quanti-iT PicoGreen assay. Irradiance measurements, including ultraviolet-C (UVC) output and maintained UVC flux of the devices, were recorded using an integrating sphere. The data were analysed using descriptive and inferential statistics.

Results: There was no difference between the mean concentration of the DNA extracted from the aqueous and air samples (p = 0.3494). An accumulation of TB DNA copies/m^3 with increasing time, when the devices were OFF, was observed as expected. Forty-six percent (6 of 13) of UVGI devices tested yielded 100% effectiveness in a controlled laboratory setting; 5 of 6 had built-in fans which may have contributed to their efficacy. The effectiveness of the remaining devices ranged from 43.7% to 95.1%.

Conclusion: The efficacy of UVGI devices available in South Africa is highly variable, with minimum UVC output. The reduced levels of effectiveness of some devices might be due to the design of the devices, which needs to be reassessed by manufacturers. The effectiveness of UVGI devices and quantification of microbial survival rate can be assessed robustly using qPCR.

Keywords: TB, engineering controls, airborne infection control, healthcare workers, health facilities

INTRODUCTION

Occupational tuberculosis (TB) remains a major hazard for healthcare workers (HCWs) globally, and particularly in South Africa, as they are inadvertently and inevitably exposed to the bacillus when interacting with untreated or ineffectively treated patients.[1-3] The high rates of TB, multi-drug resistant TB (MDR-TB) and extreme drug resistant TB (XDR-TB), combined with the co-epidemic of human immunodeficiency virus (HIV) and deficiencies in infection control practices, increase the burden of disease.[4,5] The incidence of TB among South African HCWs was 860 per 100 000 in 2013.[6] HCWs are up to six times more likely to be hospitalised for DR-TB than the population for which they care, the incidence rate ratio is 5.46 for MDR-TB and 6.28 for XDR-TB.[1] Claassens et al. showed that the occupational TB risk for HCWs at primary healthcare facilities was similar to that for hospital-based HCWs.[7]

The challenge of addressing behavioural issues and the realisation of how TB can change one's life irrevocably has accelerated efforts to ensure that HCWs are adequately protected from this preventable disease.[8,9] Retention of skilled HCWs is crucial for the implementation of programmes that address the burden of the co-epidemics of TB and HIV. Airborne diseases such as TB, legionellosis and aspergillosis are also of concern for social and economic reasons.[10] The high relative risk to allied HCWs further underpins the need for interventions to limit occupational exposure,[11] by advocating for more effective airborne prevention measures to create safer working conditions in healthcare facilities.

Airborne transmission of TB strains is the most common route of infection[1] as droplet nuclei can remain airborne for several hours.[12] It is generally believed that hospital-associated transmission of TB

from patient to HCW is the most likely mode of transmission due to poor or non-existent prevention and control measures,[13,14] and that this occurs even in non-clinical areas.[13,15]

The high mortality from the Tugela Ferry TB outbreak in KwaZulu-Natal in 2006[16] emphasised the role of mechanical engineering in infection control. Dilution-mixing ventilation is a common means of reducing airborne transmission.[17,18] However, it becomes unfeasible and unsustainable at high healthcare facility occupancy rates, and is progressively less effective at reducing risk as each doubling of the airflow reduces the risk of infection by approximately 50%.[19] In many instances, facilities cannot be readily re-engineered to maximise ventilation. Therefore, ventilation must be evaluated using validated measurement tools and, if found to be inadequate, must be supplemented with other preventive measures. However, the dire need to prevent airborne transmission with supplemented engineering controls such as UVGI is hindered by the lack of evidence-based efficacy assessments. Whilst UVGI should not be considered as a primary infection control strategy, it may be installed if the ventilation is inadequate (i.e. less than 80L/s/person in high-risk areas),[3,20] if the facility cannot be re-engineered to maximise ventilation, and/or if high-risk activities cannot be relocated.

Scientific evidence underpins the theory that well-designed UVGI devices have the potential to reduce airborne TB bacilli.[21-23] The bacteria are inactivated by ultraviolet C (UVC) light (typically 254 nm) emitted from the UVC lamps of these devices. The susceptibility is dependent on the UV dose (fluence rate) and differs by microbial species. Upper-room UVGI devices are configured to inactivate airborne contagions in the upper part of the room while minimising radiation exposure to persons in the lower part of the room.[17,24,25] Although there has been renewed interest in UVGI, its performance characteristics in occupational settings are poorly defined. Gaps in dosing strategies, related to lamp or device efficiency, have been considered in recently developed dosing strategies.[18]

Tests to assess the efficacy of UVGI devices are not standardised but, in general, the objective of the UVGI system design is to reduce airborne transmission by more than 80%.[18] This can be achieved using three recommended approaches: i) performance modelling, using computer-aided design (CAD) or computational fluid dynamics (CFD) software applications; ii) measuring the effective Clean Air Delivery Rate (CADR) using either a constant decay or constant generation method in a high-containment bioaerosol chamber with fully mixed air[26]; iii) design using the total maintained radiant flux (independently measured in an integrating sphere) to achieve a volumetric flux density of at least 15-20 mW/m^3 for the room. The approach will depend on the availability of data and design tools.[27] Therefore, testing of devices using the biological contaminants of interest should be considered, rather than relying on design equations[23,28] and modelling tools to determine performance which has equipment, software and implementation limitations.[29]

The choice of analytic method to determine microbial inactivation is important. Preconceived acceptance of culture-based techniques as the gold standard is unfounded. Culturability has a number of limitations, for example, stress from length of aerosolisation. It is also well known that culture techniques underestimate the number of bacterial counts as many organisms are viable but non-culturable for a number of reasons.[30] In addition, the sampling process in itself damages or kills a percentage of the organisms, further underestimating the count. Quantitative polymerase chain reaction (PCR) is a good alternative to culture for the rapid and specific detection and enumeration of bioaerosols, in particular, TB. The 16S rRNA gene in TB is an appropriate target for TB identification and quantification purposes as there is only one copy in the bacterium's genome.[31] PCR detects DNA of both viable and non-viable bacteria and therefore provides a better approximation of total number of bacteria aerosolised versus the percentage inactivated after UVGI treatment. Determining the precision and accuracy of the extraction of the DNA, and accurate quantification of the bacteria, will not only demonstrate optimal efficiency of DNA recovery, but will dispel misnomers of PCR and reluctance to implement the technology.

However, a concern when dealing with bioaerosols is that the volume concentrations of airborne biological agents (10^3-10^6/m^3 air) are lower than those measured in aquatic and terrestrial systems (10^{12}-10^{14}/m^3 water) due to processes that are growth independent (e.g. resuspension and deposition). Therefore, it is necessary to know the analytical variability of DNA concentration to demonstrate statistical rigour of the method for estimating exposure, and for delineating the experimental differences observed in aerosol processes.

This study aimed to demonstrate the optimal efficiency of TB DNA recovery from aqueous and air samples, and to determine the efficacy of local UVGI devices for inactivating airborne TB cells (MTB H37Ra). Data on UVGI device installations in public sector health facilities were also obtained and collated to determine the coverage of installations.

METHODS

A two-phased approach was adopted, using an experimental design, followed by a mapping exercise to indicate where UVGI devices were installed in the country.

Phase I: Experimental design for microbiocidal and irradiance efficacy testing of UVGI devices

As precision often varies with analyte concentration, repeatability and in-house reproducibility were calculated by using DNA extracted from a bacterial suspension of an estimate 1 x 10^8 cells/ml of TB in aqueous and air samples. The following methods and instruments were used for the DNA extraction efficiency (a), UVGI efficacy experiments (b), and UVGI irradiance measurements (c):

a) DNA extraction efficiency

Culture strain: The laboratory used an avirulent strain of TB (*M. tuberculosis* H37Ra (ATCC 25177)) obtained from the American Type Culture Collection for all experiments as it is considered a lower-level hazard.[32]

DNA extraction and recovery: Briefly, an estimated cell concentration of the culture strain was prepared, based on a 0.5 McFarland standard which is equivalent to an estimate of 10^8 cells (Densitometer DEN-1B). Stripping solution (2 ml) was added to the aqueous (100 µl) and air samples (PTFE filters), followed by 100 µl of the lysis solution (Roche Amplicor specimen preparation kit, Germany). After lyses, the Quanti-iT PicoGreen assay (Thermo Fisher Scientific) was used to determine the efficiency of the DNA extraction from aqueous and air samples.[33] The assay uses an ultrasensitive fluorescent nucleic acid dye to quantify double-stranded DNA (dsDNA).[34] The fluorescence is directly proportional to the amount of DNA present in the sample. The concentration of the dsDNA in the unknown samples is extrapolated from a lambda standard curve of known concentrations, i.e. 1000 ng/ml, 100 ng/ml and 10 ng/ml, according to the manufacturer's instructions. The samples were analysed to determine the fluorescence, using the spectrofluorometer (FLX800) at excitation 485/20 nm and emission at 528/20 nm. The fluorescence values obtained were used to extrapolate the DNA mass from the prepared standard curve. For repeatability, DNA extraction was done in triplicate per sample and the PicoGreen dye assay was performed in duplicate, for all aqueous and air samples.

The DNA extraction efficiency (EE) was calculated using the following formula:

$$\text{DNA EE (\%)} = \frac{100 \times \text{measured DNA mass recovered (pg)}}{\text{theoretical DNA mass (pg)}} \quad [33]$$

Where theoretical DNA mass was calculated as:

$$\text{DNA mass (pg)} = \frac{[\text{genome size (bp)}]}{[0.978 \times 109 \text{ bp}]} \quad [35]$$

bp: base pairs
pg: pico gram
genome size of TB ≈ 4411529 bp[36]
1 pg of DNA (content) = $0.978X10^9$ bp[35]

b) UVGI efficacy testing

UVGI devices: The percentage effectiveness of the UVGI devices was tested using a known concentration of ~1 x 10^6 TB bacilli/ml for a period of 60 minutes of continuous aerosolisation. Airborne TB inactivation experiments were conducted in a conditioned walk-in test chamber (56 m^3) that represented ambient temperature and relative humidity, controlled at 21-24 °C[37] and 50-60%[38], respectively. The effectiveness of UVGI performance is dependent on both parameters.[28,39,40] The test chamber was temporarily fitted with upper-room UVGI devices from four major current suppliers in South Africa. Thirteen UVGI devices, which included ceiling, wall, corner and portable devices (i.e. louvered and closed devices that could be free-standing or mounted), were tested at a height of 2.1 m from bottom of device to floor. This placement created a band of UVGI in the upper level of the room, with an average band height of ~30 cm. The portable devices were placed on a benchtop. The devices were challenged independently with a known concentration of TB H37Ra culture in the walk-in test chamber. The ages of the UV lamps of the devices were unknown at the time of sampling.

Bacterial generation and collection (constant generation): A known concentration of TB H37Ra strain (range: 1.03 x 10^6 - 6.86 x 10^6 of TB DNA copies/ml) was prepared in sterile water containing 0.05% Tween[20] to avoid clumping, and was aerosolised using a 6-jet Collision Nebulizer (SKC, USA) and medical oxygen (O_2) (Afrox, SA) at 40 psig or 270 kPa. The nebuliser was positioned at a height of 0.9 m, mimicking the average height of a hospital bed. The uniformity of aerosolised bacterial mixing was achieved with a ceiling-mounted paddle fan (132 cm) set on medium (approximately 170 rpm). In addition, air samples were collected from the centre and from each of the corners of the test chamber to ascertain the stability of aerosolised bacterial concentrations. A coefficient variation of 15% was obtained for airborne TB concentrations at different positions in the chamber (data not shown). Air samples were collected using 1.0 µm polytetrafluoroethylene (PTFE) filters (SKC, USA), and high-volume sampling pumps (SKC, USA) at 20L/min. The PTFE filters were stationed in the centre of the chamber at a height of 1.5 m above the floor. The sampling was performed for 60 minutes with the UVGI devices set at OFF and ON. The test chamber was decontaminated with a dilution of 70% Isopropylene (Germstar), 2% Ultraseptin, 5% sodium hypochlorite and 70% ethanol between the testing of the different devices and between the ON and OFF settings. Sterile water was tested to rule out any contamination between ON and OFF sampling; the results were all negative. The sampling pumps were calibrated using a TSI4100 series flow meter (TSI Instruments Ltd, UK) for quality control purposes; if the flow rate was within 5% of the initial sampling flow rate, the sample was considered acceptable.[41]

Mycobacterium detection: The DNA from the air samples was extracted and analysed using a quantitative real-time polymerase chain reaction (qPCR) method (LightCycler Mycobacterium Detection Kit (Roche, Germany). An Amplicor respiratory specimen preparation kit (Roche, Germany) was used to extract the DNA. The qPCR method uses the Mycobacterium genus specific primers KY18 (5' – CACATGCAAGTCGAACGGAAAGG-3') and KY75 (5' –GCCCGTATCGCCCGCACGCTCACA-3') to define a sequence of approximately 200 base pairs within this region. These two primers hybridise to regions conserved among the TB complex, and amplify regions of the 16S ribosomal RNA target gene. The TB 16S rRNA gene was selected as it is a good target for identification and quantification purposes, because there is only one copy of this gene in the bacterium's genome.[31] TB DNA is sensitive to UV irradiation as the photons form thymine dimers, rendering the DNA strand unreadable, and therefore cannot be amplified or detected.[42] The DNA samples were analysed in triplicate using the LightCycler 1.5 (Roche, Germany), and the average DNA concentration (DNA copies/ml) was calculated.[43] Negative and positive laboratory and kit controls were included in each test run. The tests were only accepted if the acceptance criteria were met, as per the standard

operating procedure, and if the efficiency of the standard curve used to extrapolate the concentrations of the unknown samples was ≤ 2, as recommended by the kit manufacturer (Roche, Germany). The total airborne concentration (Mycobacterium DNA copies/m^3) was calculated using the number of Mycobacterium DNA copies/ml, the sampling time and the flow rate. The results obtained represent the total number of TB DNA copies, where one DNA copy represents one TB bacillus.

c) Irradiance measurements

Irradiance measurements of the UVGI devices were conducted at the Light and Vision Laboratory of the University of Pretoria. Measurements included standard lamp reference values, input power (rate of electrical energy consumed by device measured in watts (W)), output UVC (the instantaneous rate of total radiant energy emitted from the device in the UVC spectrum measured in W), total maintained UVC flux (the rated minimum total output of UVC of a device expected after lifetime depreciation), eye safety irradiance, maximum UV (germicidal) irradiance, power efficiency (i.e. output UVC/input UVC), and maximum air movement through the enclosed device. The most important characteristic to be determined for the radiation source (the device) prior to calculations is its spatial radiant intensity distribution. This characterisation is performed using a gonioradiometer which provides the required radiant intensities in all directions for computer-aided design (CAD).[44]

Phase II: Mapping exercise

A questionnaire was self-administered by delegated authorities of 119 healthcare facilities. All TB testing and treatment facilities listed by the provincial TB coordinators were selected. The questionnaire sought to obtain information on the selected health facility, number of UVGI devices installed, date of device installation, device model, type of device (ceiling or wall), name of supplier, maintenance record of the device, and whether the device was functioning or not.

Ethical clearance for the study was received from the University of the Witwatersrand Human Research Ethics Committee (Medical) (clearance certificate no. M120325). Perrmission was also obtained from all provincial Departments of Health.

Statistical analysis

The data were analysed using descriptive and inferential statistics, with Excel 2007. The accuracy of qPCR was determined by comparing the difference between the measured DNA value and true value of the bacteria. The precision of the qPCR was determined by the measure of relative variability of repeat samples, i.e. the coefficient of variation (CV). The means and standard deviations were calculated from duplicate samples. A two-tailed t test was performed to determine the differences between the means of the DNA concentrations when the UVGI devices were ON and OFF, at the 95% significance level.

RESULTS

The mean concentrations of DNA extracted from aqueous and air samples did not differ significantly ($t = 0.9682$; $p = 0.3494$). The repeat measurements under unchanged conditions produced similar results, which demonstrates the precision of the DNA extraction of the air samples. The coefficients of variance (CV) of the replicates of the test samples of the extracted TB DNA of 1 x 10^8 cells/ml were less than 10% (Table 1).

For efficient bioaerosol collection, the difference between the measured value (DNA mass) and theoretical value is related to the efficiency of the DNA extraction from cells of aqueous and air samples. TB DNA extraction efficiency of the aqueous samples was measured as

Table 1. Repeatability results showing DNA concentration of aqueous and air sample extracts of five independent experiments (in triplicate)

Sample	Aqueous samples DNA concentration (ng/ml)			Air samples DNA concentration (ng/ml)		
	Mean	SD	CV (%)	Mean	SD	CV (%)
S1_E1	91.84	1.65	1.8	66.46	0.64	1.0
S2_E1	104.05	2.03	2.0	64.35	1.39	2.2
S3_E1	69.67	3.85	5.5	82.17	0.59	0.7
S1_E2	79.27	0.00	0.0	72.45	3.22	4.4
S2_E2	55.40	1.61	2.9	69.04	3.21	4.7
S3_E2	65.63	6.43	9.8	61.08	1.61	2.6
S1_E3	98.50	0.81	0.8	64.31	4.03	6.3
S2_E3	68.87	0.81	1.2	88.24	0.81	0.9
S3_E3	116.16	3.22	2.8	77.99	2.42	3.1
S1_E4	137.57	1.59	1.2	107.80	7.15	6.6
S2_E4	108.92	0.80	0.7	104.99	1.59	1.5
S3_E4	82.51	3.18	3.9	91.50	1.59	1.7
S1_E5	78.20	0.00	0.0	113.57	2.86	2.5
S2_E5	84.77	3.57	4.2	96.89	2.14	2.2
S3_E5	99.93	10.72	10.7	93.36	4.29	4.6
Average	**89.42**	**2.68**	**3.0**	**83.61**	**2.50**	**3.0**

S = sample; E = experiment, SD = standard deviation; CV = coefficient of variation

Table 2. DNA extraction efficiency between aqueous and air samples

Experiment	Average DNA recovered (pg)		DNA extraction efficiency (%)	
	Aqueous sample	Air sample	Aqueous sample*	Air sample†
E_1	531113	425960	117.7	80.2
E_2	400605	405152	88.8	101.1
E_3	567062	461091	125.7	81.3
E_4	657987	608563	145.9	92.5
E_5	525759	607640	116.6	115.6

The DNA extraction efficiency (EE) was calculated using the following formulae:

$$*\text{DNA EEaqueous (\%)} = \frac{\text{measured DNA mass recovered (pg) x 100}}{1 \times 10^8 \text{ TB cells X } 4.510^8 \text{ x}10^{-3}\text{pg/cell (theoretical mass of TB genome)}}$$ [33]

$$^{\dagger}\text{DNA EEair (\%)} = \frac{\text{measured DNA mass recovered from air (pg) x 100}}{\text{measured DNA recovered from aqueous solution (pg)}}$$ [33]

the ratio of extracted TB DNA per cell to the theoretical DNA mass per cell, and ranged from 88.8% to 145.9%. The DNA extraction efficiency of the air samples was measured as the ratio of extracted TB DNA mass per cell in the air samples to the mass of the DNA per cell in the aqueous samples, and ranged from 80.2% to 115.6%. The reason for some values being greater than 100% may be attributed to clumping of TB cells in the aqueous sample. The difference between the means of the DNA masses in the aqueous and air samples values was not statistically significant ($t = 1.9278$; $p = 0.1261$) (Table 2).

The effectiveness of UVGI devices ranged from 43.7 to 100% (Table 3). The highest percentage survival of TB bacilli was 56.3% for a portable UVGI device, which indicates that the device was ineffective.

The reduction in mean DNA copies/m^3 observed between ON and OFF test runs for all devices was statistically significant ($t = 5.2837$; $p = 0.0003$). Table 3 shows that the UVC output ranged from 126 to 1900 mW; the maintained UVC flux was variable between the devices, ranging from 0.11 to 1.62 W. The power efficiency ranged from 0.15 to 2.78%. Device H, with the lowest maintained UVC flux, was neither the most nor the least power efficient. Very few open and closed UVGI devices were designed to be eye safe (i.e. not cause eye irritation, conjunctivitis or keratitis) in accordance with long-standing national and international standards.[45] The UVGI exposure dose in occupied rooms, measured with a calibrated cosine corrected UVC radiometer, should not exceed 6 mJ/cm^2 for mercury vapour lamps at 254 nm.[46]

There were challenges in obtaining information from health facilities and suppliers regarding the numbers of UVGI devices installed across the country. A total of 15 098 device installations across South African health facilities was reported by health facility managers or equivalent in the period February to October 2014 (Table 4). The information provided, regarding installed UVGI devices, was poor; one supplier reported installing 17 000 devices in the same period. Two provinces could not provide installation information. KwaZulu-Natal reported that UVGI devices were installed in two hospitals but were discontinued after receiving health complaints (e.g. skin irritation and conjunctivitis). In the 11 facilities in Gauteng that confirmed the operational status of 5 731 installed UVGI devices, 62% (3 532) of the devices were either not functional or not in use.

The mapping exercise revealed that devices are installed in many facilities with little consideration of the requirements of air mixing, room volume, occupancy for a dosing strategy and routine maintenance. There was no evidence of operation and maintenance manuals or training records of operation and maintenance at the time of data collection for all devices. In two hospitals in Gauteng, 169 UVGI devices were installed in 2007 but were not maintained. Several companies cannot be traced by the health facilities for maintenance, replacing or disposing of defective devices as reported by the facility authority. In addition, the facilities lacked evidence of effectiveness of the devices installed. Although most suppliers had a SABS certificate (SANS (IEC) 60 598-2-1) for their devices, this certification is for general purpose luminaires and does not cover the effectiveness of the device in reducing airborne contagion.

DISCUSSION

Our results for DNA extraction recovery compare with the 80% to 115% DNA extraction efficiency of whole cells from different air samples reported by Hospodsky et al., 2010.[33] The variability of DNA extraction efficiencies could be due to the loss of cells during natural decay or from bacterial cell clumping. The DNA loss may be recovered by optimising the sample collection efficiency and the DNA extraction process. The mean DNA concentrations of the aqueous and air samples were similar, showing the degree of closeness of the measured value to the theoretical value. Assessing the efficacy of airborne infection control interventions in reducing TB bacilli, using quantitative real time polymerase chain reaction (qPCR), circumvents the challenges of loss of culturability due to environmental and sampling stresses such as fluid shear stresses, relative humidity, temperature, oxygen and ozone concentration, and electromagnetic radiation.[30,47]

Table 3. UVGI device irradiance measurements and effectiveness over a one-hour sampling period, using the constant generation method

Device ID	Input Power (Apparent) (V×Amp)	Output UVC, Radiant Flux (mW)	Maintained UVC flux (W)	Power efficiency (%)	TB DNA copies/m3		% Survival[§]	UVGI % effectiveness[‖]
					UVGI OFF[†]	UVGI ON[‡]		
E : portable*	28.3	NT	NT	NT	1.09E+06	6.13E+05	56.3	43.7
F : portable	77.6	740	0.63	0.81	1.96E+06	5.57E+05	28.4	71.6
I : wall	545.0	958	0.81	0.15	1.27E+06	2.68E+05	21.1	78.9
A : corner	20.3	139	0.12	0.58	3.45E+06	5.83E+05	16.9	83.1
B : wall	40.6	213	0.18	0.45	3.79E+06	6.01E+05	15.9	84.2
C : ceiling	58.0	1900	1.62	2.78	8.05E+05	8.55E+04	10.6	89.4
G : ceiling	109.0	1035	0.88	0.81	3.12E+06	1.53E+05	4.9	95.1
D : long	NT	NT	NT	NT	1.02E+06	0.00E+00	0.0 [¶]	100.0
H : wall	33.4	126	0.11	0.32	1.35E+06	0.00E+00	0.0 [¶]	100.0
J : portable*	79.6	NT	NT	NT	6.76E+05	0.00E+00	0.0 [¶]	100.0
K : portable	98.1	958	0.81	0.83	9.70E+05	0.00E+00	0.0 [¶]	100.0
L : ceiling	44.3	306	0.26	0.59	1.01E+06	0.00E+00	0.0 [¶]	100.0
M : ceiling	40.7	318	0.27	0.66	1.02E+06	0.00E+00	0.0 [¶]	100.0

NT: not tested; *Closed device; V-volt, Amp-ampere, W-watt, mW-milliwatt; [†]Cells accumulated after one hour post a 30 minute conditioning period of the test chamber; [‡]Cells accumulated after one hour post a 30 minute conditioning period of UVGI device; [§]Percentage TB bacilli survival = (1-(UVGI off - UVGI on/ UVGI off)) x 100; [‖]Percentage TB bacilli effectiveness = (1-(UVGI on/UVGI off)) x 100; [¶]No accumulation of cells after 60 minutes

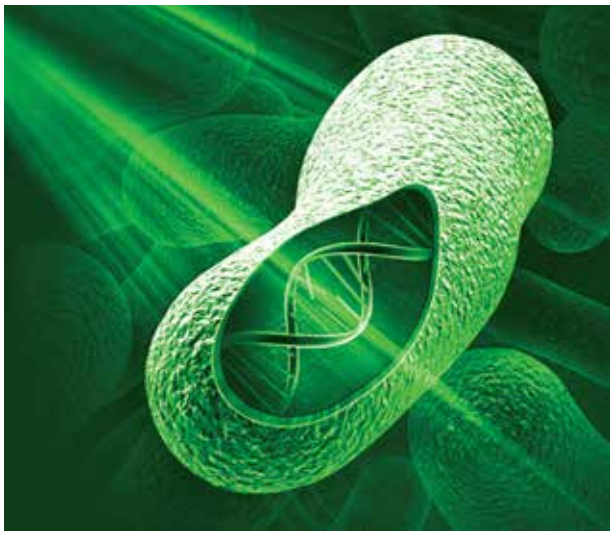
Six of the 13 UVGI devices (46%) tested met the desirable effectiveness of inactivating TB bacilli by 100% in the controlled laboratory setting. One of these six devices was an open un-louvered device with a 140 Watt UVC lamp and cannot be compared to the other devices. Eighty percent (4/5) of the remaining effective devices contained built-in fans which could have contributed to the device effectiveness in the confined testing walk-in chamber. This finding is supported by other studies where such devices have been shown to be effective in small rooms.[18] The effectiveness of the other devices tested ranged from 44% to 95% which is lower than the required six-log reduction in microbial population principle for sterilisation, or the D99 principle where UV dose results in 99% disinfection rate.[42] In addition, many of the devices with 100% performance are not readily installed in the healthcare facilities, based on the mapping exercise, as these were closed devices as opposed to louvered devices.

The irradiance of locally-supplied UVGI devices was variable, producing orders of magnitude of lower UVC output than the electrical power consumed by the open devices. This explained the lack of effectiveness of many of the tested devices. UVC lamps are typically about 30% efficient at converting electrical input power into output UVC irradiation.[48] The survival probability of bacteria after UV irradiance depends on the susceptibility of the target microorganisms and the dose of UVC to which they are exposed.[24] The devices with closely-spaced louvers were less effective than the closed devices, possibly due to a reduction in the UV output. The device design is functionally critical as high UV output is required in the upper room and low output in the lower room so as not to compromise workers and public safety. These observations are corroborated by an assessment, by the Council for Scientific and Industrial Research (CSIR), of UVGI devices installed in health facilities of the Tshwane district in Gauteng province, where fluence rates > 30 μW/cm^2 measured at 1 m from the device were demonstrated in only 26% (25/97) of devices.[49] This raises concerns about maintenance of installed devices in health facilities. Quarterly evaluation of the output UVC is recommended to assess continued effectiveness over the lifespan of the bulb.

The study results demonstrate several barriers to effective implementation of UVGI systems, justifying the moratorium on new installations in South African public health facilities imposed by the National Health Council. The dissolution of the moratorium on new UVGI installations in public health facilities rests on the manufacturers and suppliers, which must assure the quality and efficacy of UVGI devices to end users. The mapping exercise highlighted poor to no maintenance of UVGI devices and, since the lifespan of a UV light bulb is approximately 8 000 hours (~one year), the majority of devices would be ineffective in reducing the airborne TB concentration after this period. This creates a false sense of security amongst HCWs as the devices are ineffective if not maintained, despite producing a "blue light".

Table 4. Number of UVGI devices installed in health facilities across seven South African provinces

PROVINCE	No. devices
Gauteng	8 472
Limpopo	3 674
North West	1 778
Western Cape	771
Eastern Cape	224
Free State	104
Northern Cape	75
Total	15 098



Other performance aspects such as training of staff, and decommissioning and disposal of non-functional devices, were also given little or no attention. It is pertinent to evaluate the performance and cost-effectiveness of UVGI devices before procurement and installation; cheaper devices may not necessarily offer good value for money as the solution also depends on the number of devices required per area. The ratio of the volume of the irradiated zone to the entire room greatly affects the efficiency of the system.[17] It is imperative that the specifications of UVGI devices are obtained from the suppliers to enable the evaluation process. While some suppliers had conducted microbiological assessments, the sampling methodologies applied were inappropriate for airborne disinfection. Some assessments were also done[42] prior to engineering enhancements to devices, without re-testing.

A limitation of this study was the application of the continuous generation method for aerosolising TB bacilli, as the percentage effectiveness achieved only applies to the laboratory setting or test chamber, and hence, is not applicable in the field due to several uncontrolled variables. The expectation was that the performance be > or = 99.9% for all devices in the laboratory; performance is tested with TB bacilli in sterile water, which lack a mucous coating, hence the experimental organisms would be more susceptible to the UVGI devices compared to droplet nuclei from an infected person. UVGI device irradiance and percentage effectiveness were not correlated (data not shown) due to the fact that the efficacy of devices is dependent on other factors, including airflow, thermal gradients, humidity and natural die-off.[50] Studies have reported that the greater the UV fluence rate in the irradiated zone, the more effective the system is, until an upper threshold is reached upon which increasing UVGI does not increase the system's ability to inactivate microorganisms further.[24] This also indicates that effectiveness of a device determined in a laboratory setting will need to be scaled for factors of real-world installation settings, such as room volume and environmental conditions. These combined scaling factors may differ by installation, confounding their application if installation design is not done properly.

Previous studies assessing dosing strategies of UVGI devices did not take device efficiency into account. The recent UVGI NIOSH guideline lacks a standardised method to measure the upper-room fluence rates.[18,23,28] Applying the concept of one 30 W UV lamp or two 15 W UV lamps for every 19 m^2 of floor area in crowded conditions, one UVGI device for every seven room occupants may underestimate the protection as a lot of UV irradiation is lost when exiting the device.[51] Instead of using average UV fluence rates for a device's UV horizontal plane or irradiated zone, the average fluence rate for the entire room volume is a preferable method as it is adaptable to different facility settings, provided the device characteristics are provided.[18,52] The lack of knowledge of how the technology works and how to size these systems in different settings is one of the major shortcomings of poor device performance.[53] Providing dosing strategies will drive manufacturers to design systems to maximise effectiveness and will assist with comparable evaluations. Installation techniques vary considerably among manufacturers and currently are not regulated by a governing body to ensure proper efficacy of UVGI after installation. Steady progress has been made to accelerate efforts to fill the knowledge gaps about UVGI globally. Technical standards have been formulated in South Africa as well as an evidence-based draft guideline to aid facility personnel with various aspects (e.g. design, procurement, maintenance, disposal) of the life cycle of UVGI devices.[27,54] This guideline is informed by the challenges experienced in various health facilities, from planning to disposal of UVGI devices, and includes efficacy testing. Therefore, in many respects, it supersedes the South African Medical Research Council guide which was adapted from the Centres for Disease Control and Prevention.[27,55]

Based on the recommended WHO ventilation rate of 12 air changes per hour (ACH) for airborne precaution rooms, an adjustment for a nominal room volume of 24 m^3 yields an equivalent ventilation rate of 80L/s/person for high-risk settings.[56] This resultant value can be applied to high-risk settings with mechanical ventilation. An average of 160L/s/person is similarly recommended for application to rooms relying on natural ventilation.[27] For UVGI to be used as a supplement or replacement for ventilation, as permitted by current and emerging legislation, it must be evaluated on the basis of ventilation equivalence (American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) 62.1).[37] Reverting to the common theoretical basis for the 12 ACH criterial and adopting a per person ventilation rate as required by ASHRAE 62.1 enables this practical approach to UVGI adoption. It should also provide a tool to promote its appropriate use within the current and emerging legal frameworks, and not hinder it. The number of devices required for differing room occupancies can be calculated using the equivalent clean air delivery rate ($CADR_e$) value as opposed to the percentage effectiveness from the continuous generation method used in this study.[27] UVGI efficacy is determined by comparing the concentration of airborne bacteria with and without exposure to UVGI, using the constant generation method, whereas the decay method determines the rate at which airborne microorganisms are inactivated.[57,56] The latter methodology will be pursued in future research.

The application of UVGI as an engineering control against TB

infection transmission in healthcare facilities remains poorly implemented due to a lack of resources, lack of faith in the efficacy of the devices, and a poor understanding of the technology.[2,17] The cost benefit of UVGI is not difficult to justify given that TB may be fatal, may be transmitted to others, and has a long duration of expensive and poorly-tolerated treatment. Thus, it is imperative that efforts are made to improve airborne infection control.[58,59]

CONCLUSION AND RECOMMENDATIONS

The results of this study demonstrate that the qPCR is a robust tool for assessing UVGI effectiveness and quantifying the microbial survival rate. Fewer than half of the tested UVGI devices met the desirable effectiveness of inactivating TB bacilli by 99.9%. The local UVGI devices vary widely in effectiveness, therefore it is important for facilities to know the device UVC output and effectiveness. In addition, there is a lack of maintenance planning for UVGI devices and trained personnel to conduct routine checks of device performance. The validation results presented here illustrate statistical rigour that must accompany qPCR bioaerosol measurements. Therefore, the application of qPCR in aerosol science and engineering is expected to lead to improving the quality of assessing airborne infection control interventions like UVGI.

LESSONS LEARNED

- qPCR and air sampling proved to be a robust tool for assessing UVGI effectiveness and quantifying the microbial survival rate.
- The mean DNA concentrations of the aqueous and air samples were comparable, thus proving high recovery rates.
- The local air disinfection devices varied widely in effectiveness, ranging from 43.67 to 100%, with 46% inactivating TB cells completely.
- The devices with closely-spaced louvers were less effective than the closed devices.
- There is a lack of maintenance planning for UVGI devices in South African healthcare facilities across seven provinces.
- Monitoring performance of the UVC fixtures is essential as insufficient UVC dose per room will not provide adequate protection.

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DECLARATION

The authors declare no conflicts of interest.

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